THE BACTERIAL FLORA OF RACHITIC STOOLS.

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Rachitis appears to be intimately associated with a disturbed condition of the normal digestive powers of the child, and is in all probability caused by badly balanced or insufficient feeding. Findlay, however, traces it to a lack of exercise such as can be brought about experimentally by confinement, and his experiments are certainly very convincing. But possibly the lack of exercise brings about a digestive disturbance, especially if it is accompanied by improper feeding, and undoubtedly some of the symptoms, *e.g.*, abdominal distension, constipation, offensive motions, point to intestinal disturbance.

This being the case, it seemed to be possible that a bacteriological examination of the stools of children suffering from the malady might show some common bacterial condition, and with this idea a few specimens of dejecta were examined. The samples were not so numerous as could have been wished for the end in view, but Rachitis is not a common disease in Australia, and a greater number could not be obtained. Still enough was done to give an indication of the nature of the bacteria generally found.

The specimens of the stools* were received in sterilised bottles, to the wooden corks of which an iron spoon was attached. Upon its arrival at the laboratory, the sample was thoroughly mixed and a gram of material was rubbed up in a sterile glass mortar with 99 c.c. of sterile water. One c.c. of this was mixed with 99 c.c. of sterile water(=suspension i.). Two c.c. of this suspension were mixed with 98 c.c. of sterile water (=suspension ii.). Plates of media were prepared, and, after the agar had set, a diagonal was drawn across the bottom of the Petri dish with a glass pencil. A large loop of stout platinum-iridium wire (internal diameter of loop = 4·5 mm., was dipped in suspension i., and smeared over half of the plate. The loop was then pushed over

* For the specimens of rhachitic stools and for the notes upon the cases, I am indebted to Dr. Storie Dixson.

the diagonal and the second half smeared. Thus a thick and a thin seeding were obtained. A second plate was similarly treated with suspension ii. The surface moisture was then evaporated by exposing the uncovered Petri dishes in the incubator at 37° for half-an-hour, after which the plates were covered, inverted and allowed to incubate for from 1 to 3 days, the longer time being necessary for the growth of the streptococci.

The media employed were MacConkey's lactose bile salt neutral-red agar, Endo's fuchsin agar, lactose agar or nutrient agar, with the addition of either 0·1 c.c. of normal lactic acid or 0·5 c.c. of 10% sodium carbonate per 10 c.c. nutrient agar, nutrient gelatine and glucose agar for anaërobic cultivation.

Anaërobic cultivation did not assist in isolating organisms other than those which were capable of growing aërobically. The anaërobic glucose-agar plates favoured a growth of streptococci, but these were also favoured by the aërobic-acid and especially the alkaline media. *Bac. sporogenes enteritidis* was specially sought for, but was never found. Deep tubes of glucose-agar incubated anaërobically did not reveal microbes other than those obtained aërobically upon plates, and the method was rather troublesome on account of the formation of gas bubbles and an exudation of bouillon.

The colonies that developed upon the plates were examined, and probable races and species were picked out and stroked upon agar and gelatin. The condensed water of the agar cultures was examined for the motility of the organisms, and these tests, together with the morphological appearances and reaction to the Gram stain, enabled the bacteria to be thinned down to a few possible kinds. These were purified by plating on gelatin, and presumably pure colonies were picked out and stroked on agar. From these cultures, Lemco-gelatin with various sugars, etc., litmus-milk, nitrate bouillon, etc., were infected. The non-motile organisms were frequently tested for motility.

It is possible, by pursuing certain methods of enrichment, to obtain from stools a very varied flora containing perhaps *Bac. bifidus*, or one of the bacteria grouped under the name *Bac.*

acidophilus; and although it is not claimed that the analysis of the bacterial flora of the stools, as given in this paper, gives a true indication of all the kinds of bacteria present, yet they are more representative than the enumeration of a number of bacteria originally present in infinitesimal proportions and isolated after enrichment in special fluid media. Preliminary experiment had shown that the growths on MacConkey's and Endo's agar were very similar to that obtained on nutrient agar or gelatin, while the special media enabled a count to be more easily made. The comparison between the media will be seen under D.S. (p. 42).

Another thing that was brought out in some preliminary experiments was, that, to have any clear idea of the flora of the stools, it would be necessary to count the various colonies upon the plates. It appeared to be useless to indicate the kinds of bacteria isolated without at the same time indicating the relative proportions in which they occurred.

Fæcal Bacteria of the *coli*-type,
positive to indol and nitrite tests, negative to Gram, gelatine not liquefied.

No.	Motility.	Gelatin growth.	Neutral red.	Dextrose.		Mannit.		Lactose.		Milk.		Saccharose.	
				Acid	Gas	Acid	Gas	Acid	Gas	Acid	Clot	Acid	Gas
B_1	x	flat	x	x	x	x	x	x	x	x	x	x	x
B_2	x	raised	x	x	x	x	x	x	x	x	x	x	x
B_3	x	flat	—	x	x	x	x	x	x	x	x	x	x
B_4	—	raised	—	x	x	x	x	x	x	x	x	x	x
B_5	—	flat	x	x	x	x	x	x	x	x	x	x	x
B_6	—	raised	x	x	x	x	x	x	x	x	x	x	x
B_7	x	flat	—	x	x	x	x	x	x	x	x	—	—
B_8	—	flat	—	x	x	x	x	x	x	x	x	—	—
B_9	—	flat	x	x	x	x	x	x	x	x	x	—	—
B_{10}	—	raised	x	x	x	x	x	x	x	x	x	—	—
B_{11}	x	raised	—	x	x	x	x	x	x	x	x	—	x
B_{12}	—	flat	—	x	—	x	—	x	—	x	x	x	—
B_{13}	x	flat	x	x	—	x	—	x	—	x	x	x	—
B_{14}	—	flat	x	x	x	x	x	—	—	—	—	—	—
B_{15}	—	raised	x	x	x	x	x	—	—	—	—	—	—
B_{16}	x	raised	x	x	x	x	x	—	—	—	—	—	—
B_{17}	x	flat	x	x	x	x	x	—	—	—	—	—	—
B_{18}	—	flat	—	x	—	x	—	—	—	—	—	—	—
B_{19}	x	flat	x	x	x	—	—	—	—	—	—	x	x

x Positive. — Negative reaction.

Races of *Enterococcus*.

No.	Growth on agar and gelatin.	Gram.	Acid from					Milk clot.	Nitrite.	Indol.	Neutral-red.
			Dextrose.	Saccharose	Mannit.	Lactose.	Milk.				
Str. i.	scanty.	x	x	x	—	x	x	x	—	x	—
Str. ii.	scanty.	x	x	x	x	x	x	x	—	x	—
Str. iii.	scanty.	x	x	x	x	x	x	—	—	x	x

x Positive. — Negative reaction.

The physiological characters of the bacteria which have no action upon gelatine, which are negative to the Gram stain, which reduce nitrate to nitrite, and which form indol in peptone salt solution, differ so gradually from one another that it appears probable that they have been derived from a common type or ancestor, and have become altered by various conditions of environment. It appears to be simply a question of research to obtain all graduations from an absolutely positive to an absolutely negative race. It is therefore difficult to fix a race to any one name. The absolutely positive races are undoubtedly *Bac. coli communis*, but the absence of any one, or even more than one positive character, is not sufficient to differentiate the race, for the character may be only temporarily lost. Some work which is in progress upon the permanency of these race-characters shows that this is the case. One of the typical characters of a bacterium is its motility, but an absence of motility may have been noted in a faulty medium or at a wrong time. For example, B_4 was non-motile at first, but after cultivation for some weeks in the laboratory, it became actively motile. It therefore appears to be a mistake to include a race under the name *Bac. coli immobilis*. The regeneration of the one character in the case of B indicates that, under favourable circumstances, other negative characters may become positive.

For this reason, the races in the table have been classified as follows—B_1 to B_6, *Bac. coli communis*; B_7 to B_{11}, atypical *coli*; and B_{12} to B_{19}, indeterminate.

The streptococci fell into three groups, called Str.i., ii., and iii., all probably races of the same organism. No.i. is of the salivary type, inasmuch as it ferments (*i.e.*, produces acid from) saccharose but not from mannit. No.ii. is *Str. acidi lactici*, which, according to Sittler,* is identical with the Enterococcus so frequently mentioned by writers upon the flora of infants' stools.

The following are the analyses of the stools, the numbers of bacteria being expressed in percentages. The notes are by Dr. Storie Dixson (Infants' Hospital) :—

A.J., aged 12 weeks. Incipient Rickets.

MacConkey's medium ...	B_2, 100
Endo's medium	B_2, 20; Str. i. and ii., 80
Acid agar	B_2, 95; *Micr. candidus*, 5
Alkaline agar	Str. ii., 100
Glucose-agar (anaërobic) ...	
Acidity of stool	1 grm. = 0.6 c.c. $\frac{N}{10}$ acid

Typical bacterium = *Bac. coli communis.*

Typical streptococcus = Str. ii.

Notes.—Outpatient, incipient rickets, only came once. 11/8/09.

H.M., aged 1 year and 8 months. Convalescent from acute Rickets.

MacConkey's medium ...	B_1, 100
Endo's medium	
Acid agar	
Alkaline agar	Str. iii, 100
Glucose-agar (anaërobic) ...	
Acidity of stool	1 grm. = 1·5 c.c. $\frac{N}{10}$ acid (very acid with strong fæcal smell)

*Centrl. für Bakt. Orig. 47. 16.

Typical bacterium = *Bac. coli communis.*
Typical streptococcus = Str. iii.

NOTES.—Outpatient, came for treatment on 6/5/09, very characteristic shape of head, severe vomiting and diarrhœa, motion sent 19/8/09. At 17 months of age anterior fontanelle was over ½ inch wide at widest part.

L.S., aged 11 months.

MacConkey's medium ...	$B_{6,}$ 100
Endo's medium	$B_{6,}$ 30; $B_{18,}$ 5; Str. iii., 65
Acid lactose-agar	$B_{6,}$ 15; Str. iii., 85
Alkaline agar	Str. iii., 100
Glucose-agar (anaërobic) ...	
Acidity of stool	1 grm. = 0 1 c.c. $\frac{N}{10}$ acid

Typical bacterium = *Bac. coli communis* (*immobilis*).
Typical streptococcus = Str. iii.

NOTES —Inpatient, admitted 26/7/09, motion sent 19/10/09. Indications of rickets only slight.

V.B., aged 1 year and 8 months. Convalescent from slight Rickets.

MacConkey's medium ...	$B_{12,}$ 90; $B_{14,}$ 10
Endo's medium	$B_{12,}$ 19; $B_{14,}$ 1; Str. i., 80
Acid lactose-agar	$B_{14,}$ 20; Str. i., 80
Alkaline glucose-agar ...	Str. i., 100
Acidity of stool	1 grm.=0·3 c c. $\frac{N}{10}$ acid

Typical bacterium=indeterminate.
Typical streptococcus=Str. i.

NOTES : —Inpatient admitted 23/3/09, motion sent 19/10/09. Symptoms of rickets only slight.

M.E., aged 1 year and 2 months. Convalescent from acute Rickets.

MacConkey's medium ...	$B_{7,}$ 100
Endo's medium	$B_{7,}$ 88; Str. i., 12
Acid lactose-agar	$B_{7,}$ 100
Alkaline agar ...	Str. i., 100
Glucose agar (anaërobic)	

Acidity of stool ... ... 1 grm.=0·4 c.c. $\frac{N}{10}$ acid
Typical bacterium=atypical *coli*.
Typical streptococcus=Str. i.

Notes:—Outpatient, first treated 23/3/09, severe rickets, motion sent 18/8/09, by which time the severe sweats, pallor and diarrhœa had long disappeared. Though now 14 months old, was only beginning to cut its teeth (six appearing almost simultaneously). A brother, 4 years of age, had a very characteristic form of head, and the other children of a family of six had all died early in life.

D. S., aged 9 months. Pronounced Rickets.

MacConkey's medium ... B_{7}, 70; B_{19}, 30
Endo's medium ... ... B_{7}, 85; B_{19}, 12; *Bac. vulgaris*, 3
Acid lactose-agar ... ... B_{19}, 86; B_{7}, 14
Nutrient agar ... ... B_{19}, 28; B_{7}, 12; Str. iii., 60
Alkaline lactose agar ... Str. ii., 25; Str. iii., 75
Glucose-agar (anaërobic) ... B_{19}, 30; *Bac. vulgaris*, 5; Str. ii., 65
Acidity of stool ... ... 1 grm.=0·3 c.c. $\frac{N}{10}$ acid
Typical bacterium=indeterminate: *coli (immobilis)* : : 3 : 2
Typical streptococcus=Str. iii.

D.S. : three days later.

MacConkey's medium ... B_{13}, 100
Endo's medium ... ... B_{13}, 98; B_{18}, 2
Acid lactose-agar ... ... B_{13}, 50; Str. iii, 50
Nutrient agar ... ... B_{13}, 94, Str. iii., 6
Alkaline lactose-agar ... B_{13}, 50; Str. ii., 50
Nutrient gelatin ... ... B_{13}, 100

Enriched in glucose-bile salt bouillon (anaërobic) then

Endo's medium ... ... B_{1}, 50; B_{16}, 50
Nutrient agar ... ... B_{1}, 98; B_{5}, 2
Acidity of stool ... ... 1 grm.=0·4 c.c. $\frac{N}{10}$ acid
Typical bacterium=indeterminate.
Typical streptococcus=Str. iii.

Notes:—Inpatient, admitted 30/8/09, motions sent 1/9/09 and 4/9/09. Very characteristically shaped head, motions brown and relaxed, but though these rapidly lost their relaxed nature, they remained browner than usual in infants.

This is the only case where very pronounced rickets was present when the motion was sent and the patient only just beginning to be treated.

All the above infants had the characteristic head, and though two were only mild cases, the others had been fairly severe.

The bacterial flora of these stools is varied, but the relative preponderance of *Bac. coli communis* in some of the stools, and especially in the case of the infant of twelve weeks (A.J.), raised the suspicion that a *coli*-intoxication may have some influence one way or another, either in establishing or accelerating the disease. The examination, however, of stools from healthy children showed that this was not the case, as in these, the races of the *coli*-group were well represented.

The analyses of the stools of a few healthy children are as follows:—

P.H., aged 15 months.

MacConkey's medium ...	B_5, 95; B_{17}, 5
Endo's medium	B_3, 90; B_5, 5; B_{10}, 5
Acid lactose-agar	B_5, 100
Alkaline lactose-agar ...	No growth
Nutrient agar	B_5, 100
Acidity of stool	1 grm.=neutral

Typical bacterium=*Bac. coli communis (immobilis).*

Typical streptococcus=none.

A. T., aged 10 months.

MacConkey's medium ...	B_1,* 100
Endo's medium	B_1, 50; B_{10}, 50
Acid lactose-agar	B_{10}, 63; B_{11}, 7; Str. ii., 30
Alkaline lactose-agar ...	Str. ii., 100
Nutrient agar	B_{10}, 40; Str. ii., 60
Acidity	Neutral

Typical bacterium=*Bac. coli communis.*

Typical streptococcus=Str. ii.

*It is evident that B_1 was indistinguishable from B_{10} on MacConkey's medium and nutrient agar, both of which probably contained a mixture of the two races. This mixture is shown on Endo's medium.

A.W., aged 14 months.

MacConkey's medium ...	B_8, 100
Endo's medium	
Acid lactose-agar	
Nutrient agar	
Alkaline lactose-agar ...	White sarcina, 100
Acidity	Neutral

Typical bacterium=atypical *coli* : *Bac. coli communis* : : 5 : 3
Typical streptococcus=none.

S.B., aged 5 months.

MacConkey's medium ...	B_6, 98; B_4, 2
Endo's medium	B_6, 100
Acid lactose-agar	B_6, 98; B_4, 2
Alkaline lactose agar ...	No growth
Nutrient agar	B_6, 100
Acidity	1 grm.=0·4 c.c. $\frac{N}{10}$ acid

Typical bacterium=*Bac. coli communis (immobilis).*
Typical streptococcus=none.

The analyses show that the flora of the dejecta of normal children is very much the same as that of the children affected with rickets, so far as the bacteria of the *coli*-group are concerned. But there is a difference in the comparative absence of the streptococci.

These were rather conspicuous in the stools of the cases of rickets, growing even on Endo's medium, and thus in the analyses they were prominently brought before one. Such was not the case with the normal children, and indeed it is with regard to the streptococci that any difference can be found between the diseased and the healthy condition.

According to Tissier,* the *Enterococcus*, the chief streptococcus of the rachitic stools, can withstand an acidity of from 2 to 2·45, while *Bac. coli* succumbs when 1·73 is reached. From this we infer that it is capable of producing a relatively greater

* Annales de l'Institut Pasteur 19, 109.

amount of acid. In the duodenum and upper parts of the intestine of normal children the contents are slightly acid and contain coccal forms almost to the exclusion of the rod forms. Further and further down the tract, the bacilli become more and more evident until, in the rectum, the cocci are comparatively rare and the rod forms predominate (Tissier). This is rather important, and is in agreement with the bacteriological analyses of the healthy stools, in the majority of which no streptococci were found. The fact that streptococci are more in evidence in the stools of rachitic children shows that they persist for a greater distance down the tract and that they are in relatively greater numbers all along the canal. Their greater numbers indicate a more acid condition of the intestinal contents, but it is difficult to say whether they are the cause or the effect. Probably a vicious cycle has been set up.

The rod bacteria thrive well upon sugar-free media, such as nutrient agar, while the streptococci grow better upon saccharine media, and form very scanty growths in media devoid of sugar. This appears to show that in cases of rickets in which streptococci predominate, the sugar derived from the food is in excess, and the proteid is deficient in the intestinal contents. This is in agreement with the experience of medical practitioners who, in treating cases of rickets, prescribe an alteration of the diet, increasing the proteids and the fat, and diminishing the carbohydrates. The effect of the increased proteid would be to increase the relative number of the *coli*-bacteria, while the diminution of the carbohydrate would further accentuate the difference by decreasing the streptococci. The increased fat would supply the necessary energy and heat, and at the same time offer a less favourable pabulum for the growth of the streptococci.

The case in favour of the streptococci being associated with the disease is not, however, absolutely clear. Their occurrence in the convalescent cases may weaken the argument. It is true that in two of the stools they were of the salivary type, but the third contained Str. iii., which was the typical race in the instance

of pronounced rickets. There is also the occurrence of Str. ii. in one of the normal stools.

It is probable that, as in all other biological phenomena, we must take into account the idiosyncrasy of the individual, some being able to tolerate a relative excess of streptococci while others cannot.

On the whole, there is some reason for believing that the occurrence of a preponderance of streptococci in the stools of rachitic children is associated with the disease.